Summary: Induction of protective distal mucosal immunity against HSV-2 infection

Abbreviations:

Adv	Adenoviral vector
IR	Intrarectal
IVAG	Intravaginal
gB	Glycoprotein B
pfu	Plaques forming unit
HSV-2	Herpes simplex virus type 2
tw	Tissue weight
LD_{50}	50% lethal dose
vw	Vaginal wash

Materials and Methods

Animals, cell cultures and viruses

Female C57BL/6 mice were 6-8 weeks of age during immunization with Adv. Vero cells were grown in complete α-MEM media. Recombinant AdgB is an Adenovirus vector that encodes gB8, the surface protein gene from HSV. HSV-2 strain 333 was propagated and titered on Vero cells.

IR immunization and virus challenge

Mice were anesthetized with isoflorane and instilled with 50% ethanol into the colo-rectum, and kept under anesthesia for 30 min. One hour later, AdgB was IR delivered by insertion of a pipet tip into the rectum, followed by one-hour incubation period. 21 days after AdgB IR immunization, mice were IR (rectal challenge) or IVAG (vaginal challenge) challenged with HSV-2 strain 333. For IR challenge, the procedure was as the same as that for IR immunization. For IVAG challenge, mice were injected SC with 2.5 mg of progesterone (Depo-Provera) 5 days prior to administration of HSV-2. 20 μl of HSV-2 was given IVAG, followed by one-hour incubation.

Viral replication and pathology in the anal and genital tract

After IVAG inoculation of HSV-2, vaginal washes were obtained daily by pipetting twice 30 μl of PBS into and out of vaginal tract, and stored at -70°C before use. Virus shedding was determined by plaque assay on Vero cell monolayers and expressed by virus retrieved from per vaginal wash (vw) in 60 μl.

Anal or genital pathology was monitored and scored daily after HSV-2 challenge. Genital pathology was scored by a 6-point scale from 0 to 5 (adapted from Overall et al, 1975; Gallichan et al, 1998, 2001; Kuklin et al, 1998): 0, no change; 1, redness of external vagina; 2, swelling of external vagina, severe redness; 3, perineal hair loss or genital ulceration, severe swelling; 4, perineal ulceration; and 5, hind limb paralysis or death. Anal pathology was also scored on a 6-point scale based on the descriptions by Phillips et al (1998, 2000): 0, no change; 1, redness of anus; 2, swelling of anus, severe redness; 3, perineal hair loss or anal ulceration, severe

swelling of anus and perineum; 4, perineal lesion; and 5, hind limb paralysis, anal restrictions or death.

Results

Dose dependent study of IR challenge of HSV-2

To determine the LD_{50}, naive mice were IR inoculated with HSV-2 strain 333, and monitored for anal pathology (daily for the first 2 weeks). As shown in Table 1, 50% of mice died from HSV-2 IR inoculation at a dose of 2×10^4 pfu. Anal pathology developed rapidly and no mice survived when the doses were increased to 2×10^5 pfu and 2×10^6 pfu. When mice received the latter dose, which is 100-fold higher than LD_{50}, they were all paralyzed by the first week. Because this dose was highly lethal, leading to early onset of anal pathology and rapid death, it was used to challenge AdgB IR-immunized mice.

Table 1. Survival rate (%) of naive mice IR inoculated with HSV-2 (8 mice/group).

Dose (pfu)\week	1	2	3	4
2×10^3	100	75	75	75
2×10^4	87.5	50	50	50
2×10^5	87.5	0	0	0
2×10^6	0	0	0	0

Pathology and survival from IntraRectal HSV-2 challenge in AdgB IR-immunized mice

21 days after a single IR immunization with AdgB, mice were monitored for pathology and survival following a lethal IR challenge of 2×10^6 pfu of HSV-2. All unimmunized mice (n=8) rapidly developed pathology, and were unable to survive the challenge by day 7. In AdgB IR-immunized mice (n=12), 41% of mice had overt pathology and 92% survived the HSV challenge. For those mice that developed pathology, the severity of infection was less than non-immunized mice (maximum score points: 3.6±0.9 vs. 5±0.0), and external indications of infection were no longer visible by week 2, indicating the ability of immunized mice to withstand the infection at high lethal doses.

The development of genital pathology after challenge of 2×10^5 pfu of HSV-2, which is 10-fold higher than conventional dose (2×10^4 pfu), was also assessed. All unimmunized mice died within the first week of challenge, whereas 100% of immunized mice survived. Although 60% of immunized mice demonstrated overt genital pathology (3.7±0.6 vs. 5±0.0), they were also able to control the infection, characterized by regression of some mild perineal lesions.

Virus titers in vaginal washes of IntraVaginal HSV-2 challenge in AgB IR immunized mice

Previous studies have shown that virus shedding peaks at day 3 post infection. Virus shedding was compared on monolayer Vero cells by measuring plaques formed by virus obtained from vaginal washes. Three days after HSV challenge at a dose of 2×10^5 pfu, virus was detected in the samples of all unimmunized mice ($8.0\times10^3\pm3.7\times10^3$ pfu/vw, n=5). Although 80% of immunized mice (n=5) were detected positive for virus shedding, compared to those from unimmunized mice, the virus titers from these immunized mice were at least one log lower ($8.7\times10^2\pm7.0\times10^2$ pfu/vw). While all unimmunized mice retained similar levels of virus shedding until death, 40% of immunized mice were no longer positive for virus by day 5, and all were virus free by day 10.

References